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Probiotic fermented sausages: Myth or reality?

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Abstract

Experts view the development of functional foods as an opportunity for innovation for the meat industry in line with consumers' concerns on food nutrition, well being and food safety. The main microbiota of fermented sausages consists of lactobacilli, which suggests that properly characterized strains could be selected as starter cultures. The selection of strains from human origin was considered in order to promote their survival in the host. *Lactobacillus rhamnosus* CTC1679 dominated during the ripening process and temporarily colonized the gastrointestinal tract of healthy volunteers, confirming that this strain could be delivered as a potential probiotic in fermented sausages.

Keywords: LAB; *L. rhamnosus*; *L. monocytogenes*; probiotic starter cultures; low-acid fermented sausages; adhesion; colonization

1. Introduction

Meat fermentation is an ancient preservation method resulting in microbiologically safe products with particular sensory attributes and unique and distinctive meat properties. Fermented meats play an important role in many diets and are very appreciated by the consumer. They are a source of protein, fat, essential amino acids, minerals, vitamins and other functional nutrients. Currently, consumers are very conscious about their nutrition and well being, but regrettably often associate processed meat products as being high fat, high sodium chloride and cancer-promoting food and, as a consequence, ingestion of meat and meat products is avoided or limited. As an opportunity to improve this perception, in recent years, researchers and the meat processing sector have put much effort in

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developing and marketing functional meat products, i.e., foods used to prevent and treat certain disorders and diseases, in addition to their nutritional value *per se*[1].

An attractive strategy for designing functional meat products is the use of probiotic cultures. Probiotic bacteria are 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'[2]. Those beneficial health effects can be multiple and depend on strain, dose, administration method, population group and physiological state of the host[3]. Dairy products are the most commonly used food vehicles for the delivery of probiotics. They have been on the market for many years and are well accepted by consumers[4]. However, a few studies have also demonstrated the feasibility of elaborating fermented meat products with probiotic cultures (e.g.[5,6,7]).

Most of the microorganisms used for probiotic purposes belong to the *Lactobacillus* and *Bifidobacterium* genus although the probiotic nature of other QPS (with Qualified Presumption of Safety) species (e.g. *Lactococcus*, *Enterococcus*, *Sacharomyces*, *Propionibacterium*, *Bacillus*, etc.) has also been reported[8]. Lactic acid bacteria (LAB) are currently used for the production of a great variety of fermented foods derived from plant and animal products and are also usual inhabitants (true commensals or transient passengers) of the gastrointestinal (GI) tract[9]. Characteristics of probiotic bacteria include resistance to the acidic conditions of the stomach and to bile acids and pancreatin from the beginning of the small intestine. Other key traits are the production of antimicrobial compounds (e.g. bacteriocins), the adhesion to the intestinal mucosa, the non production of biogenic amines and the absence of specific antibiotic resistances[2].

The work presented here summarises the research of our group on starter cultures for fermented meat products with the final aim of obtaining functional fermented sausages with a satisfactory overall sensory quality and safety as a potential way for delivering probiotic bacteria.

2. Isolation and characterization of LAB from intestinal origin

From a total of 109 LAB isolated from infant faeces (<6 months of age) we observed that *Lactobacillus* was the most prevalent genus (48% of the isolates) followed by *Enterococcus*, *Streptococccus* and *Weissella*. At the species level, *Lactobacillus gasseri* (21%), *Lactobacillus casei/paracasei* (10%) and *Enterococcus faecalis* (38%) were among the most predominant[10]. As *Lactobacillus* is the most technologically relevant genus in fermented sausages, further selection was focused on it. RAPD-PCR allowed the discrimination of identical strains which were further evaluated for their ability to grow *in vitro* in the processing conditions of fermented sausages (presence of NaCl, nitrite and acidity) and for their functional (antagonistic activity against *Salmonella* and *L. monocytogenes*, resistance to the GI tract conditions and aggregation capacity) and safety properties (antibiotic susceptibility and tyramine production). Considering all the results, *L. casei/paracasei* CTC1677 and CTC1678, *Lactobacillus rhamnosus* CTC1679, *L. gasseri* CTC1700 and CTC1704 and *Lactobacillus fermentum* CTC1693 were selected as potential probiotic strains and their ability to lead the fermentation (9 days at 15ºC) in model sausages was tested. Despite LAB counts being similar in all batches, not all the assayed strains were competitive in that environment and only *L. casei/paracasei* and *L. rhamnosus* strains achieved 100% implantation and acidified the mixture. Thus, *L. casei/paracasei* CTC1677, *L. casei/paracasei* CTC1678 and *L. rhamnosus* CTC1679 were able to lead the fermentation and become dominant over the endogenous LAB, confirming their suitability as starter cultures.

3. Designing safe probiotic fermented sausages

When the suitability of the previously selected strains (CTC1677, CTC1678 and CTC1679) as potential probiotic cultures was assayed in nutritionally enhanced (with reduced sodium and fat content) low-acid fermented sausages (*fuets*), we observed that only *L. rhamnosus* CTC1679 was able to grow and dominate (levels *ca*. 10^8 cfu/g) the endogenous LAB during ripening in two independent trials (Fig. 1), thus being more competitive than the commercial probiotic strains *L. plantarum* 299v, *L. rhamnosus* GG and *L. casei* Shirota[11]. At the sensory level, no significant differences were observed among *fuet* type sausages elaborated with the different starter cultures regarding most of the evaluated sensory attributes. All the products recorded a satisfactory overall sensory quality without any noticeable off-flavour, and with the characteristic sensory properties of low-acid fermented sausages.

In another study performed by our group[12] the commercial probiotic strains *L. plantarum* 299v and *L. rhamnosus* GG both inoculated at two different levels, 10^5 and 10^7 cfu/g, were shown to be competitive in Spanish-type acid fermented sausages. Additionally, they had a positive effect on the hygienic qualities of the final products, preventing the growth of *Enterobacteriaceae*. Overall sensory quality of all the sausages was acceptable although both inoculum levels of GG strain and high inoculum of 299v produced a sharp decrease of the pH and limited growth of Gram-positive catalase-positive cocci, leading to a negative effect on some of the evaluated sensory attributes when compared to spontaneously fermented sausages.

Conversely, *fuet* is a typical low-caliber dry fermented sausage made with pork meat characterized by its low acidity (pH >5.3), a fact that, together with the proposed reduction of the amount of NaCl, can compromise the safety of the product as the food-borne pathogens *L. monocytogenes* and *Salmonella* could grow. To evaluate the safety of the nutritionally enhanced *fuets* manufactured with *L. rhamnosus* CTC1679, a challenge test with low levels of *L. monocytogenes* (30 cfu/g) and *Salmonella* (20 cfu/g) was carried out. *Salmonella* was eliminated from sausages and the growth of *L. monocytogenes* prevented during the maturation process. After 35 days of storage at 4ºC, *L. monocytogenes* achieved absence in 25 g of the product[13]. Accordingly, under the conditions assayed, *L. rhamnosus* CTC1679 is suitable as potential probiotic lactobacilli able to reach high levels (10^8 cfu/g) and produce safe nutritionally enhanced fermented sausages with the characteristic sensory properties of this type of products. Considering the recommended daily dosage (>10^{8-10} cfu/day[14]), the putative probiotic effect could be achieved with the ingestion of 10g of product per day, which is feasible and compatible with a nutritionally balanced diet.

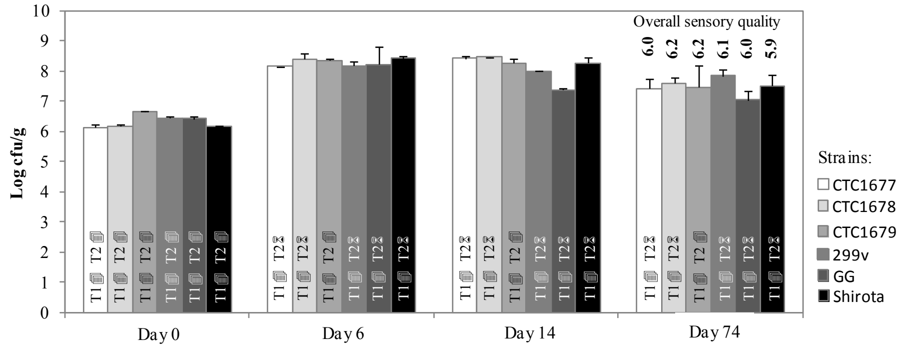

Fig. 1. LAB counts and implantation of strains during a ripening period of 14 days and a subsequent refrigerated storage of 60 days. Overall sensory quality of the final products and implantation (4) and non-implantation (6) of the strains in trial 1 (T1) and trial 2 (T2) is indicated.

4. Evaluation of adhesion and survival of probiotics in the GI tract

The *in vitro* adhesion to intestinal cell lines is often used to evaluate the probiotic potential of bacterial strains, since the transient colonization of the intestinal epithelium would allow probiotic bacteria to exert its beneficial effect. In order to mimic the colonic epithelium, the human intestinal cellular line HT29 was chosen as a model. The percentages of adhesion of *L. casei/paracasei* CTC1677 and CTC1678 were similar to those of *L. rhamnosus* GG, the reference strain used due to its good adherent properties[15] whereas the capacity of adhesion of *L. rhamnosus* CTC1679, the most competitive strain in fermented sausages, was significantly higher than that of the GG strain. Moreover, *L. rhamnosus* CTC1679, which showed the highest co-aggregation rate with the food-borne pathogen *L. monocytogenes*, was able to reduce the adhesion of *L. monocytogenes* to HT29 cells in experiments of exclusion and inhibition. Hence, this strain would be able to counteract the effect of the pathogen in the gut, a desirable characteristic for a potential probiotic strain[2].

With the aim of evaluating the survival of *L. rhamnosus* CTC1679 through the GI tract, a human preliminary intervention study was performed, given that one of the main preconditions for a potential probiotic is its ability to survive the passage through the GI tract in a number sufficient to exert a positive effect on the host. The study consisted of 5 healthy volunteers who consumed 25 g of CTC1679-fermented sausage per day for 21 days[13]. It was observed that *L. rhamnosus* CTC1679 transiently colonized the GI tract and persisted during the ingestion period at levels *ca*. 8 log cfu/g. After 3 days of non-consumption, the strain was still recovered from the faeces of all the volunteers, confirming the capability of *L. rhamnosus* CTC1679 to be delivered as a potential probiotic in fermented sausages.

5. Conclusion

The use of probiotic strains in fermented meat products represents an opportunity for innovation for the meat industry and a new way for consumers to ingest probiotics. The selection of adequate probiotic strains able to lead the fermentation process and survive both in the food product and the passage through the GI tract is crucial to obtain food products containing levels of probiotic bacteria enough to confer beneficial effects on the host.

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